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Original Research Article

An understanding of interlink of Vitamin D with Tuberculosis- A cross-sectional study

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ABSTRACT

Background: Tuberculosis (TB) is the most prevalent health disorder in developing nations and is linked with decreased immunity. Vitamin D is one of the most important vitamins required to boost immune response as it is required to maintain innate immune response via its actions on TLRs and macrophages. Risk of Tuberculosis has been associated with decrease level of vitamin D in serum in various studies. Despite of these studies some discrepancies have been shown in different studies conducted in different regions which further potentiates the role of various factors like diet, ethnicity, and seasonal variations influencing the prevalence of T.B in vitamin D deficient and non-deficient population. This study has been conducted to evaluate and establish the link of serum vitamin D in TB and non TB population residing in Mathura region of U.P.

Materials and Methods: Total studied population was 80. 40 participants were taken in patient group with newly diagnosed Tuberculosis both pulmonary and extrapulmonary and 40 were enrolled in healthy control group. Serum sample was collected twice first before the start of treatment in patient group and then after the three months of effective treatment initiation.

Results: Among the two groups i.e., control and patient serum level of vitamin D in patient group was found to be significantly low as compared to the control group (22.66 ± 15.17 vs 73.03 ± 25.6 ng/mL: $P < 0.001$). Moreover it was seen in this study that serum vitamin D level was low in extrapulmonary tuberculosis as compared to pulmonary T.B, but this is statistically insignificant ($P = 0.397$). Likewise when serum level of vitamin D was compared before start of treatment in patient group and after treatment no significant difference was seen. ($P = 0.807$). Linear regression analysis ruled out any significant link in serum levels of vitamin D when matching was done including age, gender, site of tuberculosis and after treatment onset criteria $P \geq 0.68$.

Conclusions: Healthy population has higher serum levels of vitamin D as compared to patients with tuberculosis.

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1. Introduction

Tuberculosis (TB) is one of the leading cause of death and morbidity in developing countries like India and across the world and considered as an global health issue.¹ The causative agent of this bacterial infection is Mycobacterium tuberculosis. This is an airborne infection which not only

affects quality of life but also imposes great impact on the health care system^{2,3} nearly affecting one –third population of the world, out of which approximately 10% develop active disease.^{4,5} Despite the development of advance health care system and drugs, T.B still remains the major health issues.^{6,7} Among the various risk factors which leads to generation and severity of T.B one of the most common and neglected factor is immunity. Vitamins are substances required for good health and immunity, among them the

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most important one is vitamin D required to enhance the immune response.^{8,9} Vitamin D exerts its antimicrobial activity^{10,11} by regulating macrophage function. Several studies have shown that lower serum levels of vitamin D leads to greater risk of developing tuberculosis.^{12,13} Several epidemiological studies also established the same. It was found that in winter due to low serum level of vitamin D risk of infection is more so more number of cases occurs in winter as sunlight is an important source of getting vitamin D from skin cells. Similarly elderly and patients with kidney disorders have low level of vitamin D are more susceptible for infection as compared to healthy population this also establishes the role of this vitamin in enhancing the immunity and prevention of this disease.^{14,15} Vitamin D plays important role in enhancing immunity via T cells i.e., CD4+ and CD8+ T cells, the important armour of innate immune system exerts its protective actions against TB by producing various cytokines like CC and CXC.^{8,9} Vitamin D binds to toll like receptors and exerts its antimicrobial actions.¹⁶ Vitamin D also binds to nuclear receptor in the target cell therefore deficiency of this vitamin or any other structural or functional abnormality of its receptor leads to impaired host response against tubercular bacillus.¹⁷ The active form of this vitamin 25-hydroxyvitamin D is used to determine the functional level of this vitamin.¹⁸ The antimicrobial property of macrophages requires its phagocytic activity and interferon γ which also has direct link with the serum level of vitamin D.^{12,13,19,20} Vitamin D enhances the phagocytic activity of macrophages and also results in intracellular death of *Mycobacterium tuberculosis* by producing Cathelicidin antimicrobial peptide.^{21,22} Serum Levels of vitamin D differs among individuals, have variations owing to geography, races and different culture practices.^{23,24} Therefore this study also includes these variations while evaluating the serum level of vitamin D in connection to its confounding factors.

2. Materials and Methods

This cross sectional study was done for a period of one year starting from February 2017 to December 2017 i.e., two groups were formed. One for the patients with recently diagnosed TB on treatment where treatment was initiated successfully for over three months and the other group were of healthy subjects. Ethical committee approval was taken and serum level of vitamin D in both the groups was taken. A total of 80 participants were taken in this study, 40 with the disease and 40 were healthy subjects serum level of vitamin D was measured from both the groups before start of the treatment. The patient group was given standard antitubercular treatment.

The inclusion criteria includes patients not on vitamin D supplements and having age above 20 years with newly diagnosed TB. Diagnosis confirmed by either positive smear

or culture or PCR for *M. tuberculosis* in sputum and other body specimens. Control groups comprises of individuals not taking any vitamin D supplements aged above 20 and without any clinical and pathologic, radiological findings suggestive of tuberculosis. Matching was done for age and gender for volunteers and patients. Patients and healthy subjects with any co morbid conditions and clinical conditions or on intake of vitamin D supplements or having any malabsorptive condition like celiac sprue or taking anti tuberculosis medications before were excluded from the study. Patients were informed and consent was signed both from the patients and control groups. The patients' information sheet was made which included the vitamin D levels in serum, age, gender, history of co morbidity and history of any drug usage. Serum samples were taken in the morning after overnight fasting and one before the start of treatment and one taken after three months of treatment for each patient. Whereas only one sample has been taken in the control groups. Response for the treatment was measured by clinical examination, bacteriological examination, and chest radiograph. Electrochemi luminescence immunoassay was used to measure vitamin D levels in cobas e411 analyser - Roche Diagnostics analyser. The range for this parameter has been set to 3–100 ng/mL. In this study, deficiency of vitamin D was attributed to the levels of active form of vitamin D i.e., 25-hydroxyvitamin D ≤ 19 ng/mL, and vitamin D insufficiency as 20–29 ng/mL,²⁵ mean \pm standard deviation was used to describe the data. Further different statistical parameters were used like Chi-square, Cramer's V, Pearson's and Spearman's correlation to analyse the data. Paired T-test was used to compare the two groups. SPSS software was used to analyse the results in the two groups where $P < 0.05$ was defined as a statistically significant.

3. Results

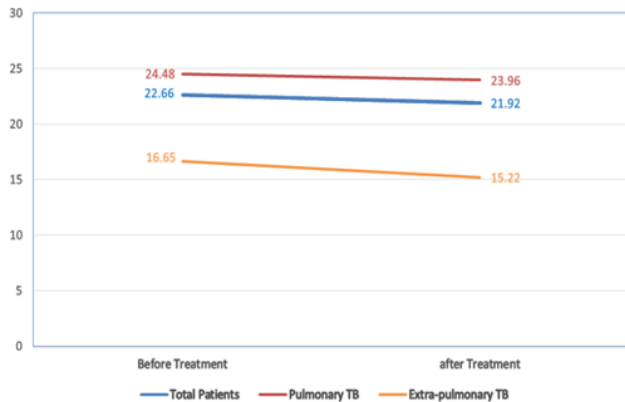
A total of 80 people have been enrolled in this study with 40 newly diagnosed patients and 40 healthy subjects. 36.5 ± 12.7 has been set as the mean age for the patients and 325.3 ± 22.3 for the healthy subjects. Each group was made with 22 men (55%) and 18 women (45%). The patient's group had 26 patients (65%) with pulmonary TB and 14 patients (35%) with extra pulmonary TB. Serum level of vitamin D was tabulated in Table 1, the mean serum level was found to be higher in healthy groups as compared to the patient group (73.03 ± 25.6 vs. 22.66 ± 15.17 ng/mL), which was statistically significant as suggested by independent T-test.

As depicted in the Table 1 the mean serum level of vitamin D was not significant before and after treatment in patients group ($P = 0.807$) and as well it was lower when compared to the control group and this difference was statistically significant in independent T-test.

No significant difference was seen in serum levels of vitamin D when confounding factors taken into account

Table 1: Patients and control groups based on serum vitamin D level.

Vitamin D status	Pulmonary TB	Extra Pulmonary TB	Control group	All
Deficiency	7(26.9%)	5(35.7%)	0	12(15%)
Insufficiency	10(38.5%)	9(64.3%)	4(10%)	23(28.8%)
Sufficiency	9(34.6%)	0	32(80%)	41(51.3%)
Potential Intoxication	0	0	4(10%)	4(5%)
All	26	14	40	80

**Fig. 1:** Vitamin D levels before and after treatment (ng/mL)

i.e., age ($P=0.297$) and gender ($P=0.182$) and site of TB ($P=0.081$) in patient group before treatment and similar results were found in patient group after treatment i.e age ($P=0.67$), and gender ($P=0.683$) and levels of vitamin D and difference was found to be insignificant. When serum levels of vitamin D was compared before and after treatment significant ($P=0.019$) and direct correlation ($r =0.452$) was found.

4. Discussion

This cross sectional study found serum level of vitamin D was higher in healthy subjects as compared to T.B patients both before start of study and after treatment. About 12.5% patients with deficiency of vitamin D and about 73% with insufficient vitamin D were reported in this study. Previous studies conducted in countries like china showed prevalence of hypovitaminosis in 83.1% among active TB patients (27) and in brazil with 75% showed hypovitaminosis also collaborates with the findings of this study.²⁶ It was found that in healthy control group only 5.8% had vitamin D deficiency. Some of the studies have shown results contrary to this like one study in Ethiopia where non T.B subjects have shown about 49% vitamin D deficiency.^{27,28} Several others studies found no significant change in patients and control groups like Koo et al.,²⁹ and Ho-Pham et al.,³⁰ Subjects with extra pulmonary tuberculosis in this study have also shown significantly low level of vitamin D as compared to healthy individuals. This is one of the few studies which has been done with cases with extra

pulmonary T.B. One with extra pulmonary T.B was done by Pareek et al.,³¹ Lower level of vitamin D was also seen in extrapulmonary T.B as compared to pulmonary T.B. This study also highlighted the fact that vitamin D level was not significantly improved after initiation of the treatment in the patient group.³² The contrary findings in different studies done in various populations across the globe can be attributed to difference in the dietary habits, % of comorbidities in the studied population, access to sunlight, seasonal effects and ethnic groups, and difference of method adopted to assess the level of vitamin D level and range taken.^{33–35} exposure to sunlight,^{36,37} the study showed no significance with the confounding factors like age and gender. Some countries like Pakistan and Ethiopia showed gender bias in level of vitamin D, where females found to have more deficient level compared to males. In contrast, several studies from Pakistan and Ethiopia showed decrease levels of vitamin D in female gender which can be ascribed to lack of sun exposure and pregnancy.^{28,38} which can be ascribed to lack of sun exposure and pregnancy. Some studies proved that ageing is associated with the decrease level of vitamin D as one study done on African population supported this hypothesis.^{28,39} The limitations of this was certain criteria's not taken into account like dietary intake clothing coverage, seasonal effects, moreover it has been conducted during the period of one year. However, this was one of the kind as it was done first time in Mathura. More researches with large populations size accounting various variables which can affect the vitamin D levels further needs to be conducted in various parts of the world to establish the role of vitamin D supplementation as preventive measure for microbial infection and also establish its importance as supplementary to the standard treatment of tuberculosis.

5. Conclusions

This result of this study can be summarised into two broad headings:

1. Serum level of vitamin D was significantly lower in patients group as compared to healthy subjects before start of treatment and after treatment initiation.
2. No significant difference in level of vitamin D was seen in patients group before and after treatment initiation.

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8. Conflicts of Interests

No conflicts of interest was there among authors.

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